

AMENDMENTS TO THE SPECIFICATION

Please replace the paragraph bridging pages 1, 2 and 3 of the specification with the following paragraph:

The regulatable promoters in *S. pombe* include the *fbp*, *inv1* and *nmt1*. The *fbp1* promoter is repressed by 8% glucose and derepressed in 0.1% glucose plus 3% maltose (Hoffman and Winston, 1989, Gene 84: 473-479). A limitation of this promoter is that it is derepressed in stationary phase and furthermore, the cells expressing the vector do not grow well under the inducing conditions. A similar drawback is faced by the *inv1* promoter derived from the *inv1* gene that codes for invertase in *S. pombe* (Tanaka *et al.*, 1998, Biochem. Biophys. Res. Commun. 245: 246-53). It is also repressed by glucose and derepressed by depletion of glucose. The regulation of expression using this promoter cannot be very tight because of progressive depletion of glucose in the culture medium as a result of its utilization during the cellular growth. Likewise another inducible promoter of *S. pombe* acid phosphatase structural gene (*pho1*), which is induced by low concentrations of inorganic phosphate in the medium, has the drawback that it shows a significant level of uninduced transcription, thus negating its potential use as a promoter (Maundrell, 1990, J. Biol. Chem. 265: 10857-64). Thus, ~~till date to date~~ only one promoter element has come to be used regularly as a research tool, namely *nmt1*, which is repressed by high concentration of thiamin and induced by absence of thiamin (Maundrell, 1990, J. Biol. Chem. 265: 10857-64). The most common and the strongest form of this promoter is *nmt1*. A few derivatives of the *nmt1* promoter were subsequently developed that yield very high (*nmt1*), medium (*nmt41*) and low (*nmt81*) levels of expression (Forsburg, 1993, Nucleic Acid Res. 21: 2955-56). A related problem of these promoters is their leakiness even under repressed conditions and the leakiness appears to be directly proportional to the promoter strength

(Forsburg, 1993, Nucleic Acid Res. 21: 2955-2956). The induction regime involves growth of cells harboring the plasmid expressing a particular gene under the control of the *nmt1* promoter in presence of thiamin. After growing to early log phase (OD₆₀₀ of ~0.3), cells are washed with and transferred to a synthetic medium lacking thiamin and grown further. The expression of the gene of interest is observed after nearly 18-20 hours of growth in the medium lacking thiamin. Apart from the cumbersome problem of handling cells under sterile conditions through the steps of washing and resuspension in thiamin-free medium, the other major problem with this promoter is that it is leaky, that is, the expression of the gene is never completely repressed in the presence of thiamin. This can lead to a deleterious effect on the growth rate of cells even before the start of induction because of possible metabolic load. A similar effect may be exerted during induction because of the long time of induction to achieve full expression level. The presence of the heterologous protein during the long induction period in the intracellular milieu may also lead to cellular defect and protein degradation. The present invention therefore obviates these drawbacks and through the process of this invention a temperature sensitive (or regulated) promoter based vector for expression of heterologous proteins in fission yeast,

Schizosaccharomyces pombe has been developed. This invention is particularly useful in efficient, economic and regulated expression of proteins both homologous and heterologous.

The new promoter elements are isolated by screening of promoters which allow expression of a Green fluorescent protein (GFP) reporter gene in response to a shift in temperature. The promoter elements thus isolated represent a truncated region of the previously reported no-message for thiamine 1 (*nmt1*) promoter (Maundrell, 1990, J. Biol. Chem. 265: 10857-64) having some unique properties that make them more advantageous to use as compared to other known promoters including *nmt1* in *Schizosaccharomyces pombe* (*S. pombe*).

These characteristics are: i) temperature sensitive expression: induction of expression by shifting the temperature from 36°C to 25°C, ii) faster kinetics of expression, iii) moderate level of expression, iv) low leaky expression and v) lack of toxicity.

**Please delete the paragraph at page 26, lines 11-36, which starts with
“INFORMATION FOR SEQ ID No: 1.”**